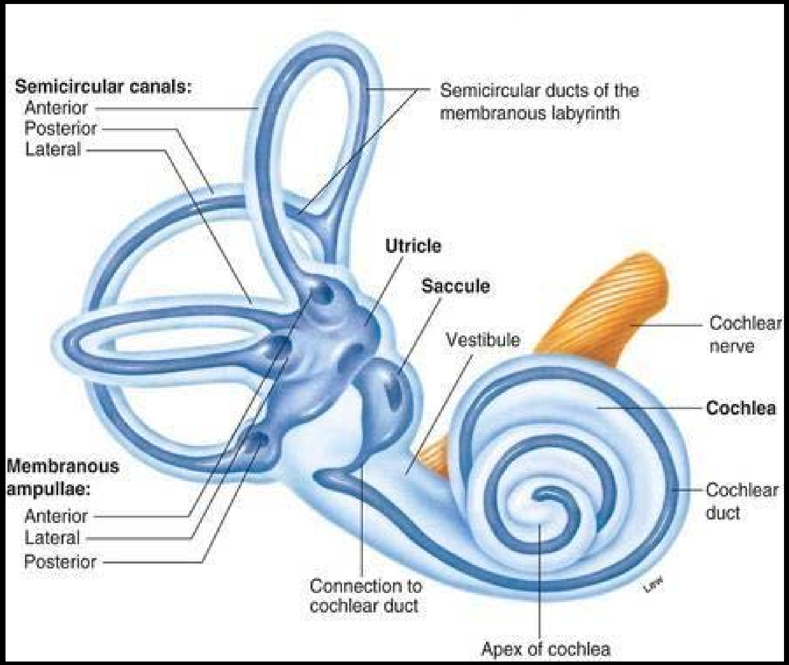
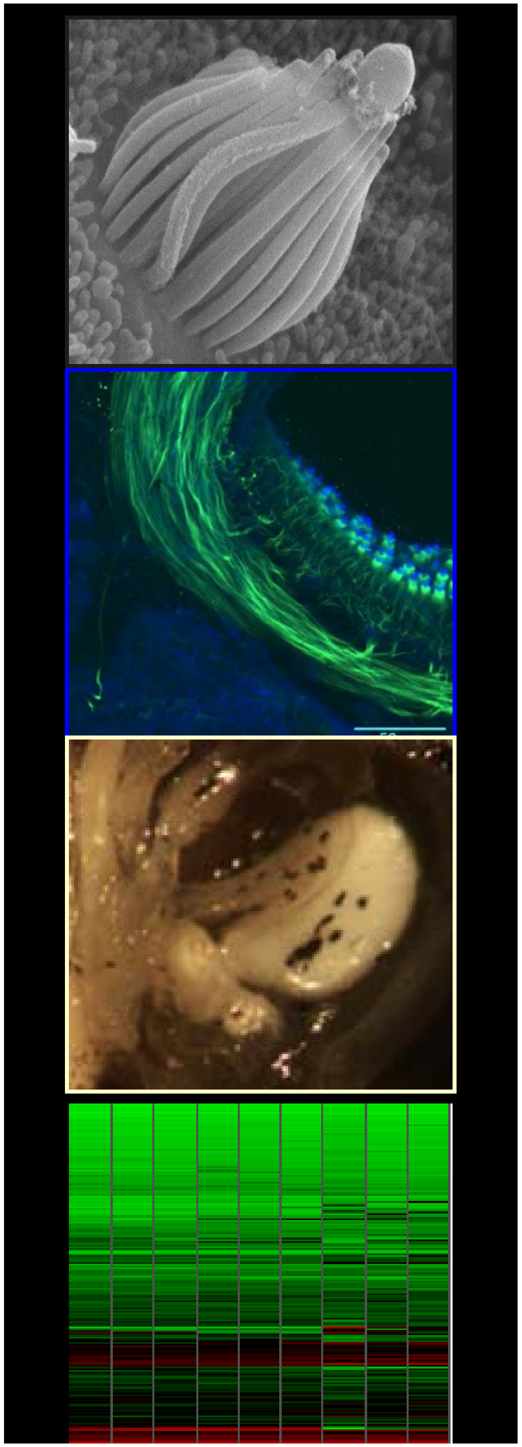
February 25, 2017
Biology Leadership
Community, Tucson

A Dancer's Delight: Organ Systems of the Inner Ear and the Senses of Hearing and Balance

Elba Serrano
Regents Professor
New Mexico State University

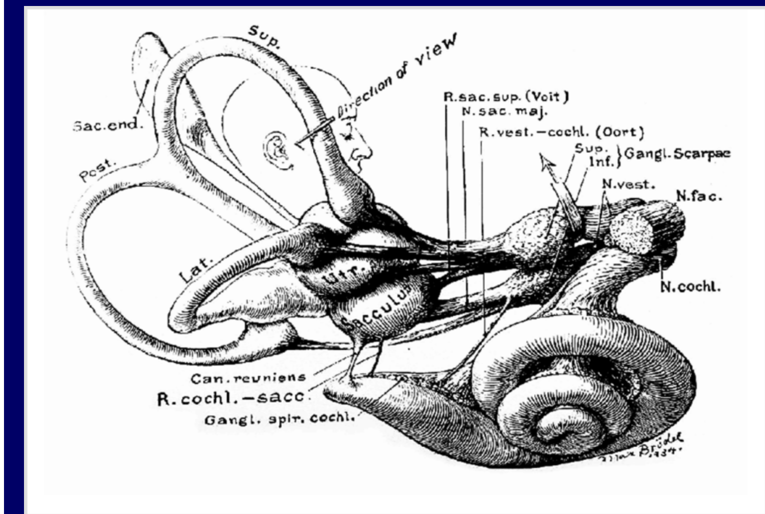


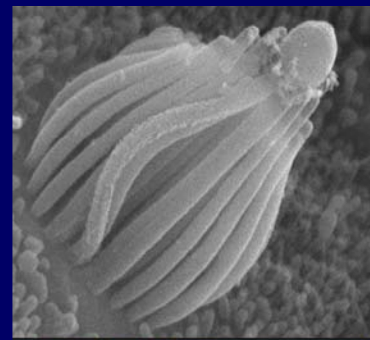
Which sense would you be willing to give up?

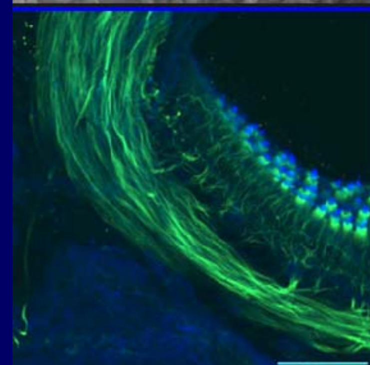


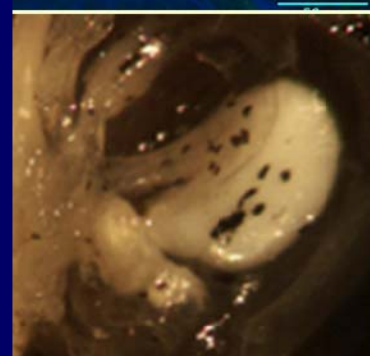
Phenotype:
Hearing and Balance

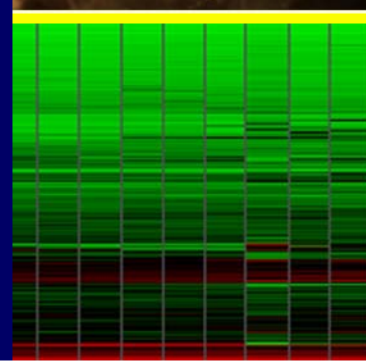
System Component:
The Inner Ear

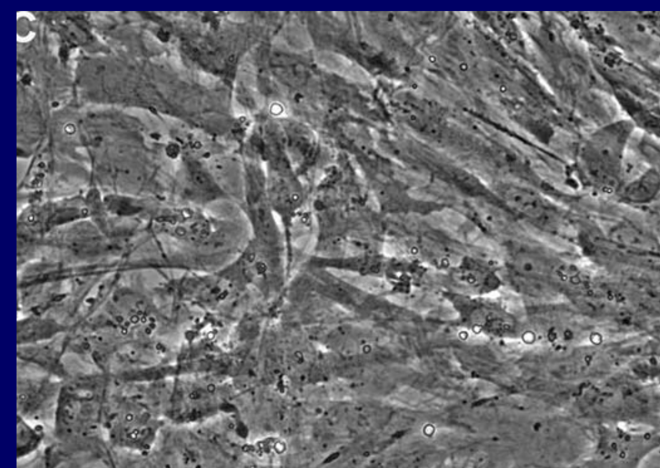

Sup.
direction of view
Sac.end.
Post.
R.sac.sup. (Voit)
N.sac.maj.
R.vest.-cochl. (Oort)
Sup.
Inf.
Gangl. Scarpae
N.vest.
N.fac.
Lat.
N.cochl.
Can. reuniens
R.cochl.-sacc.
Gangl. spir. cochl.







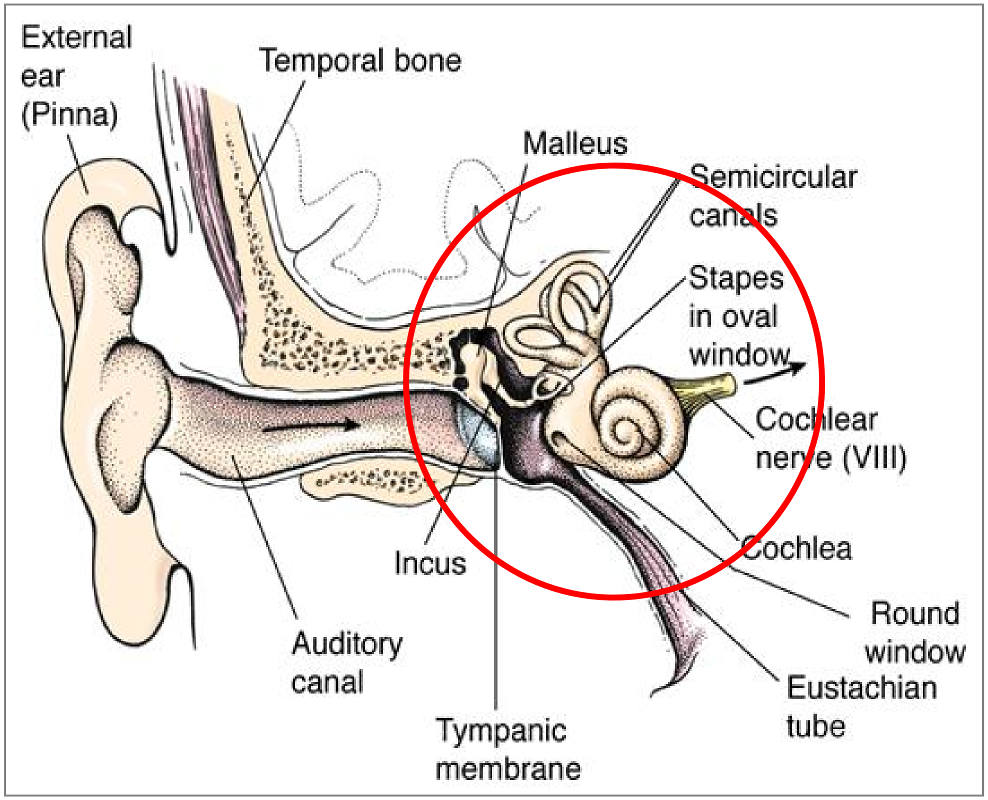




Introduction

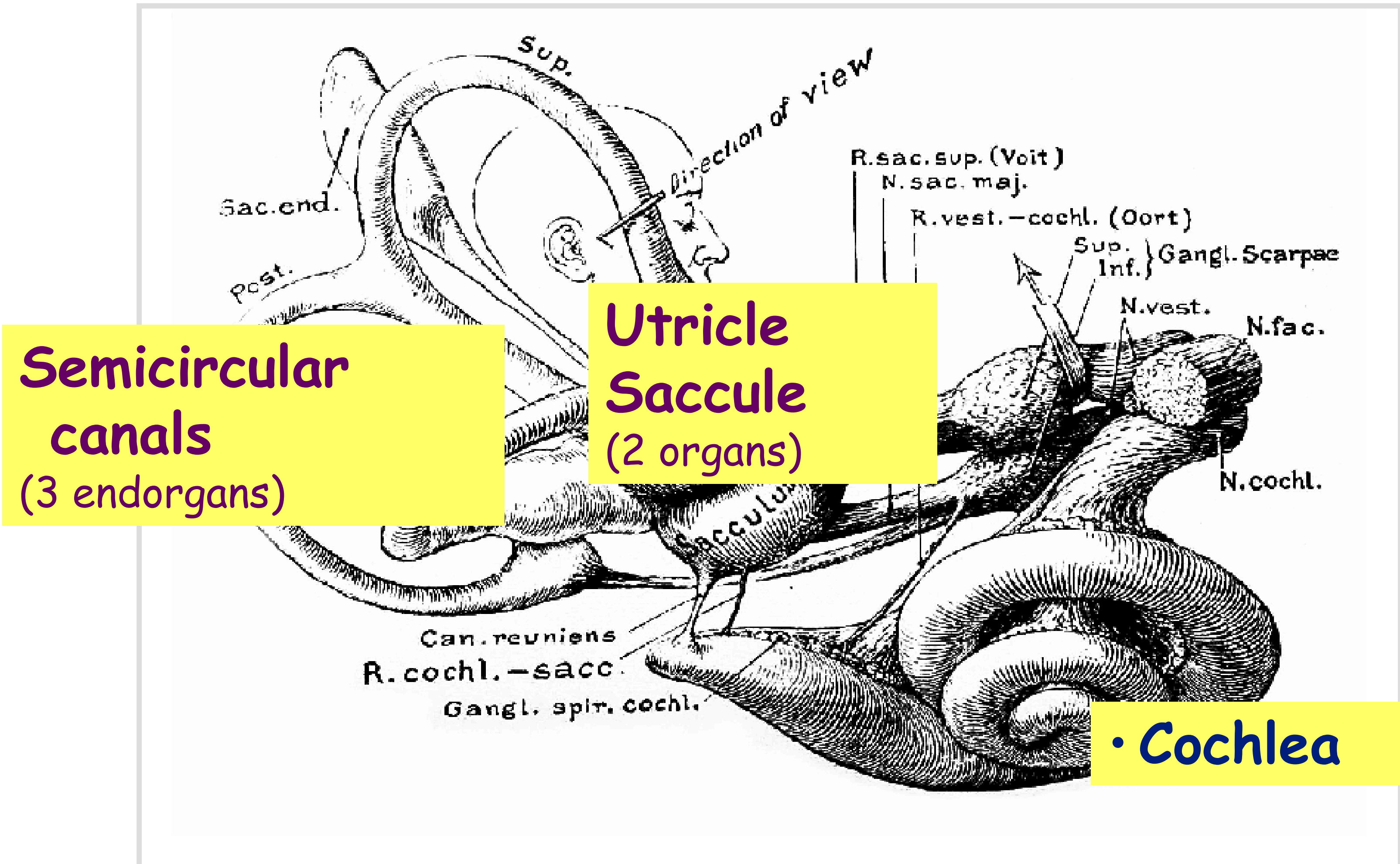
5 Core Concepts:
Auditory And Vestibular Sensation
(Hearing, Balance, Orientation)

1. The inner ear functions as a SYSTEM for *force reception*.

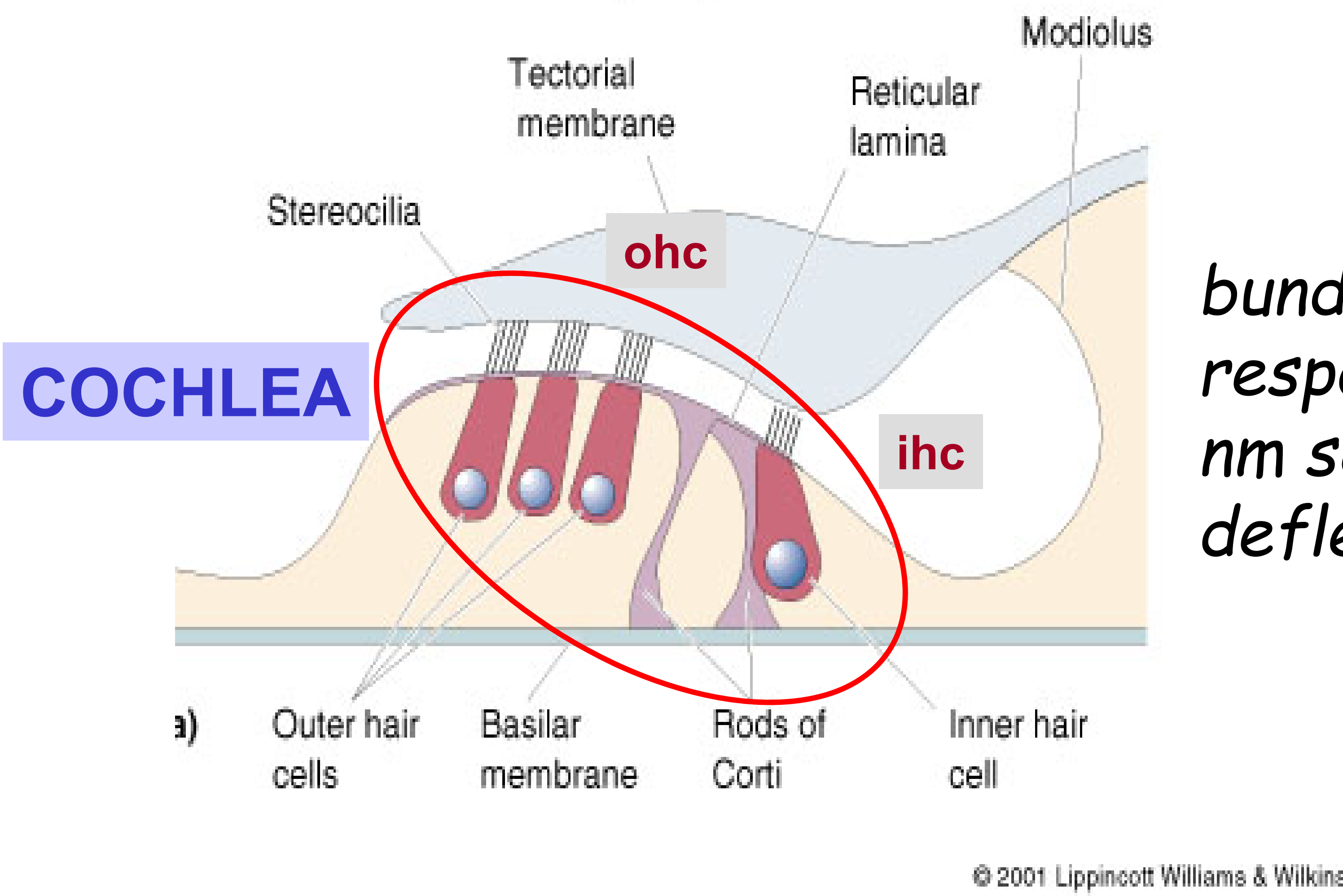


- ***Complex tridimensional organization***
- ***unique materials composition (tissues, crystals, bone)***
- ***protected by one of the hardest bones in the human body (temporal)***
- ***Force perception*** **requires *innervation (neurosensory reception)* and maintenance of *fluid balance* (endolymph-labyrinths)**

2. The inner ear organ comprises multiple smaller *vestibular* and *auditory* sensory endorgans, each with characteristic morphology and frequency selectivity.



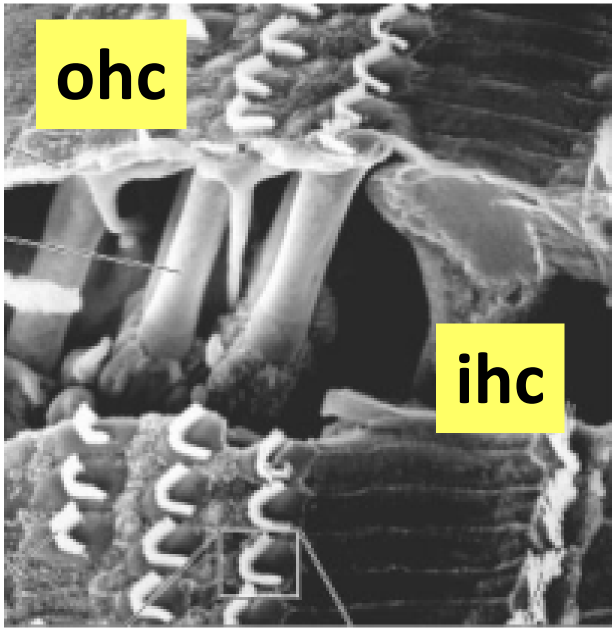
3. Specialized *mechansosensory "hair" cells* in epithelia of different sensory endorgans detect force (pressure) with *apical bundles* and relay sensory information to the CNS



bundles respond to nm scale deflections

4. Inner ear mechanosensory hair cells (MHC) are *neuroactive* and *few* in number

- **apical sensory bundles are villi and kinocilia *NOT* "hairs"**
- **MHCs are electrically active (ion channels in bundle and body) and release neurotransmitters**
- **~ 16-20 thousand auditory hair cells/human cochlea vs 100 million photoreceptors/eye**



- **~30% of MHCs damaged by age 65**
- ***do not regenerate in humans, can regenerate in fish and amphibians like Xenopus***

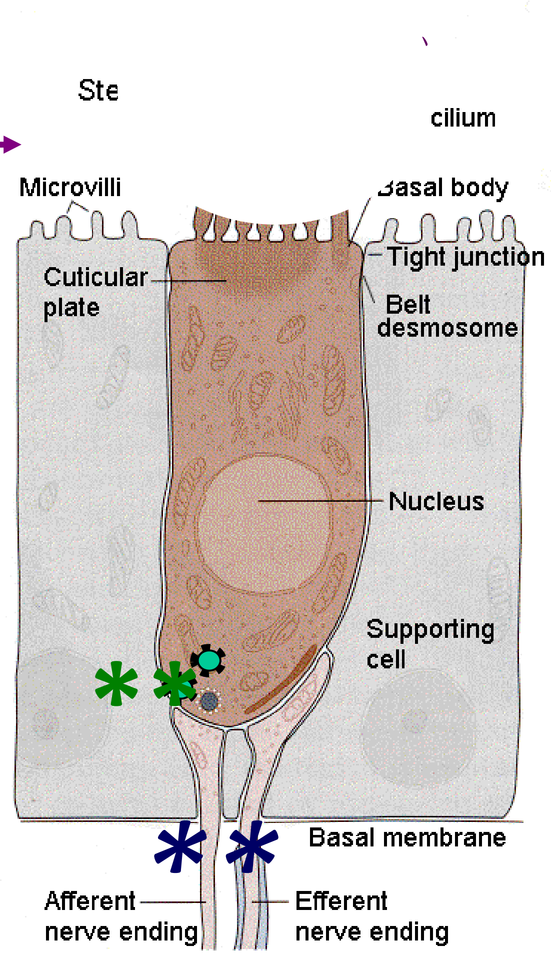
5. Mechanosensory hair cells transduce environmental forces (*sound, gravitational*) into electrical signals

bundles deflected by forces

transduction of force reception

ion flow through membrane channels

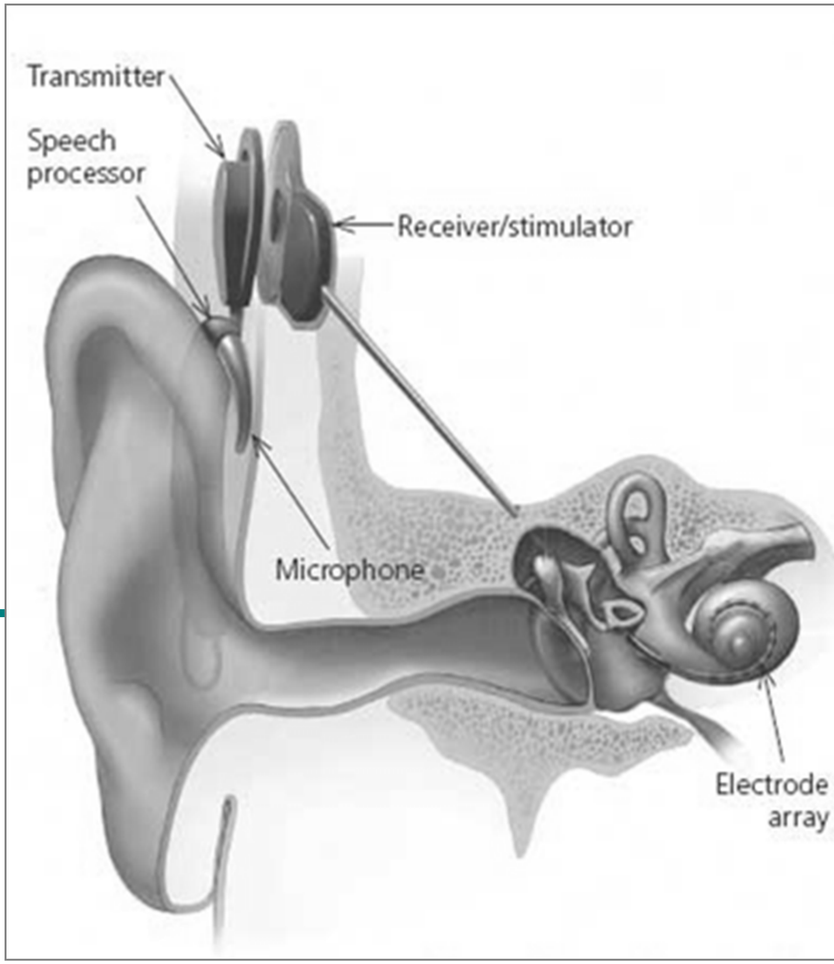
neurotransmitter release at synapse



http://www.unmc.edu/Physiology/Mann/pix_4b/hair_cell.gif

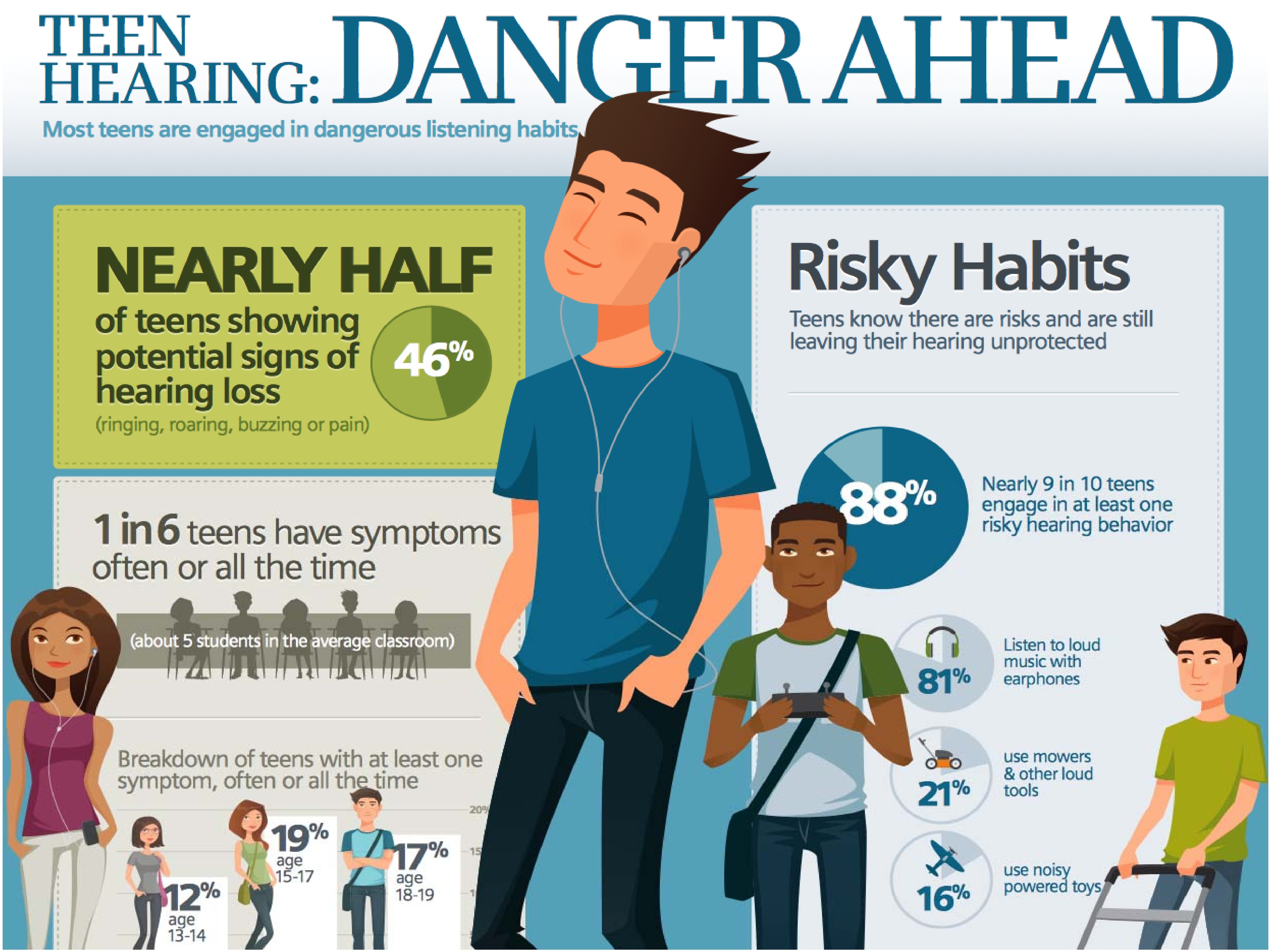
damage to bundles causes loss of hearing and balance!!

We have a special interest in the electrophenotypic differentiation of hair cells during development



DISORDERS OF HEARING AND BALANCE

Ear with Cochlear implant.
Credit: NIH Medical Arts


TEEN HEARING: DANGER AHEAD
Most teens are engaged in dangerous listening habits
NEARLY HALF
of teens showing potential signs of hearing loss
(ringing, roaring, buzzing or pain)
46%
1 in 6 teens have symptoms often or all the time
(about 5 students in the average classroom)
Breakdown of teens with at least one symptom, often or all the time
12% age 13-14
19% age 15-17
17% age 18-19
Risky Habits
Teens know there are risks and are still leaving their hearing unprotected
88%
Nearly 9 in 10 teens engage in at least one risky hearing behavior
81%
Listen to loud music with earphones
21%
use mowers & other loud tools
16%
use noisy powered toys

IMPACT: **sensorineural hearing loss is the most common sensory disorder**

- hearing impairment increasing at a dramatic rate: *worldwide over 300 million – a global epidemic*
- vestibular disorders are also debilitating (perhaps more so than deafness).....
  - *vestibular sense less understood and studied*
- **in humans, if mechanosensory hair cells of the inner ear are lost or damaged, function is essentially impaired forever**

http://www.who.int/mediacentre/factsheets/fs300/en/

National Academy of Sciences
Five Main Causes of Hearing and Vestibular Loss

1. Infections. bacterial meningitis, rubella (German measles).
2. Acoustic trauma exposure to loud sounds, noise
3. Presbycusis, aging: repeated acoustic trauma, hardening of microscopic blood vessels in the inner ear
4. Heredity. 100+ hereditary syndromes, many mitochondrial
   - 2-3 out 1000 newborns profoundly deaf
   - connexin 26 screening
   - impact on learning - essential to identify impairments in children -
5. Prescription drugs, streptomycin, tobramycin, chemotherapeutic agents (cisplatin).

CAREER TRANSITION

POSTDOC

TO

FACULTY

I ♥ ION CHANNELS

The Journal of Neuroscience, Novem

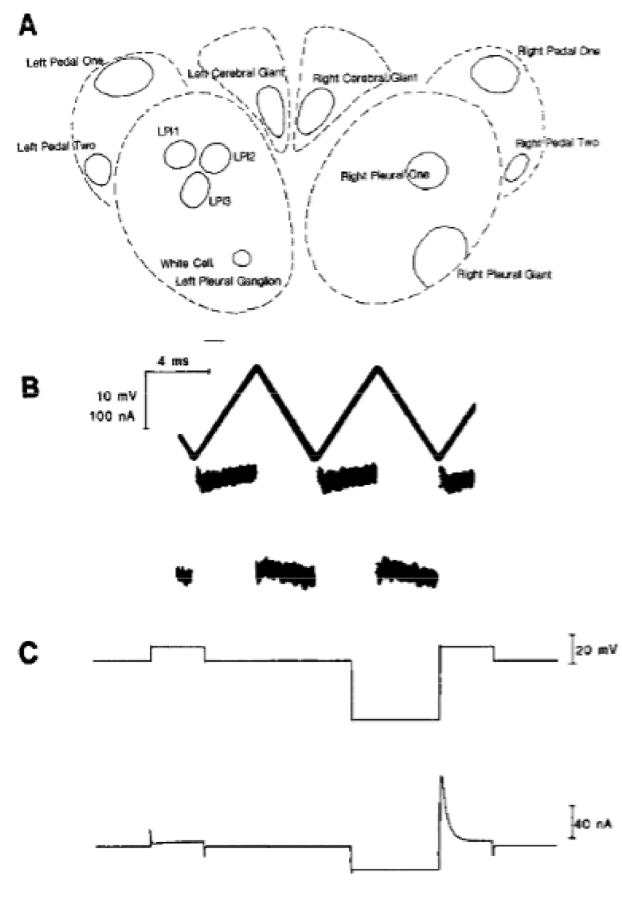
Diversity of the Transient Outward Potassium Current in S Identified Molluscan Neurons

Elba E. Serrano and Peter A. Getting[a]

Department of Biological Sciences, Stanford University, Stanford, California 94305

We have undertaken a quantitative study of the differences in the properties of the fast transient outward current (A-current) between identified neurons of 2 species of nudibranch mollusc. Somata from identifiable neurons of *Archidoris montereyensis* and *Anisodoris nobilis* were isolated and voltage-clamped with a 2-microelectrode voltage clamp at 11°C. We examined diversity in the expression of the time- and voltage-dependent properties of A-current by measuring the following parameters: (1) current magnitude, (2) current density, (3) inactivation kinetics, (4) the voltage dependence of steady-state activation and inactivation. We first characterized A-current in each cell type by measuring these parameters for each identified neuron in a series of

Voltage-clamp studies of animal and pla strated the presence of numerous ionic c derlie a wide variety of electrical proper membranes. Ion channels may be disting their voltage and time dependence, thei ion selectivity, and, in some instances, intracellular factors (Hille, 1984). The te tegration of the currents flowing through in the characteristic electrical activity of a of excitable cells, such as neurons, the repetitive firing properties emerge from the activation or expression of multiple ion channels. Differences in the classes of channels, their relative contribution to total current flow, as well as changes in their time-

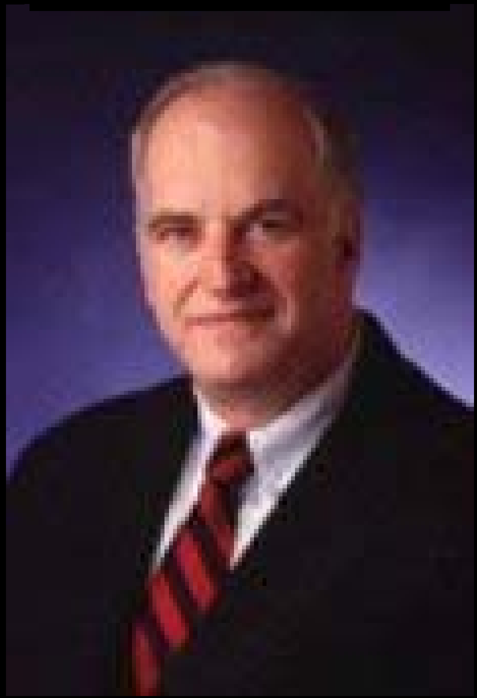


STANFORD

MENTORS

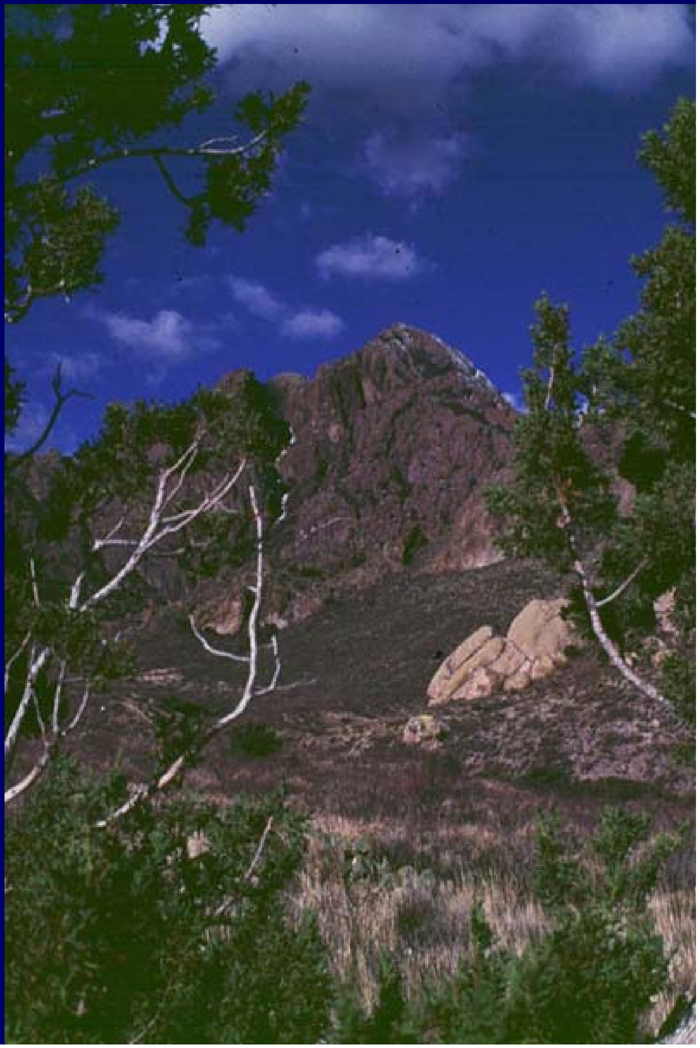
PETER GETTING SUSUMU HAGIWARA BRUCE RANSOM PETER NARINS

UCLA



NMSU Las Cruces, New Mexico

a Hispanic serving minority land grant institution with an engineering and agriculture mandate for the state



Research that contributed to basic science and relevant for hearing and balance disorders

Mechanosensory Hair Cell Development

Sensory Organogenesis

What experimental animal model?

- Suitable for facilities
- Inexpensive
- Developmental

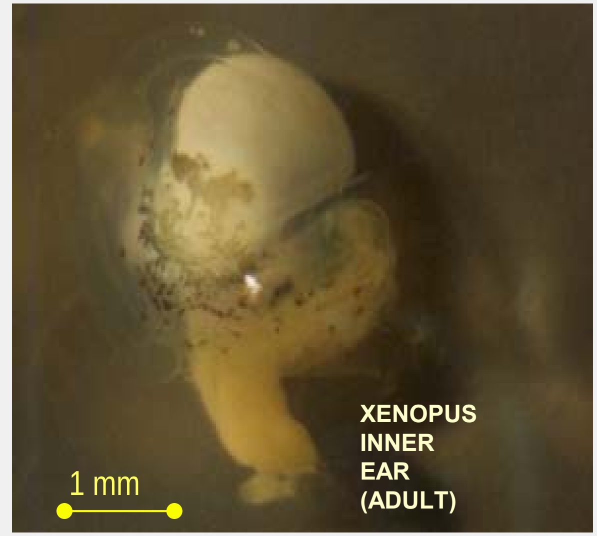

XENOPUS

XENOPUS *a.k.a.South African clawed frog/toad*

- **Human ears difficult to obtain**
- ***No mouse facilities at NMSU***
- **Non-mammals can regenerate hair cells**
- **Amphibians classical system for hair cell biophysics**
  - ***Xenopus*** **- NIH model organism (*tropicalis* and *laevis*)**
    - **Developmental stages well characterized**
      - **External fertilization**

2011 National Xenopus Resource at Woods Hole transgenic methods & molecular genetics possible CRISPR - TALEN - mutagenesis

XENOPUS INNER EAR



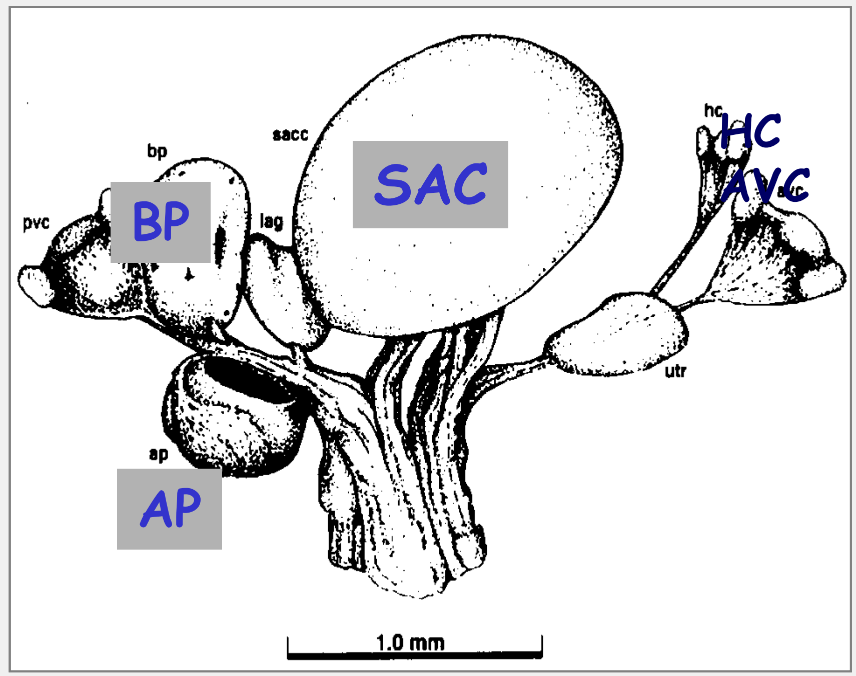
Eight sensory organs tuned to different frequencies

VESTIBULAR ORGANS

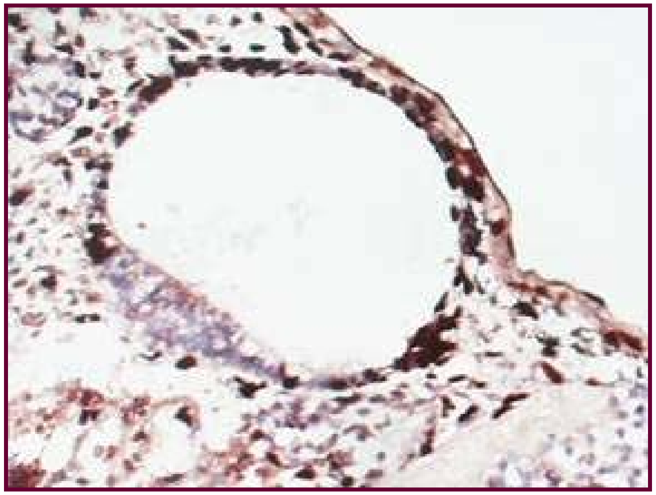
anterior, horizontal, posterior
ampullae
lagena
utricle
sacculus*

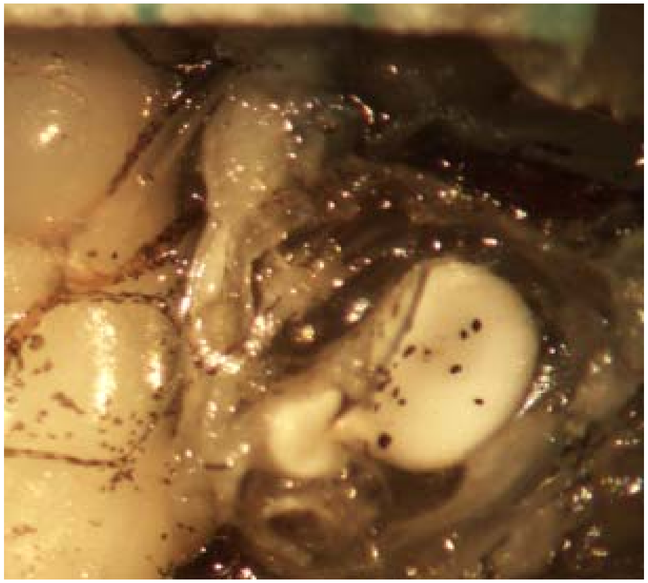
AUDITORY ORGANS

sacculus* ~ 300Hz
amphibian papilla* 300-1500Hz
basilar papilla* >1500Hz



Sensory Organ Formation
Mechanosensory Hair Cell Development





RESEARCH VIGNETTES

1. ***What are the structural phenotypes within sensory organs during development?***
2. ***Can we identify genetic targets for interventions that promote restoration or repair of damaged hair cells?***

STUDENTS

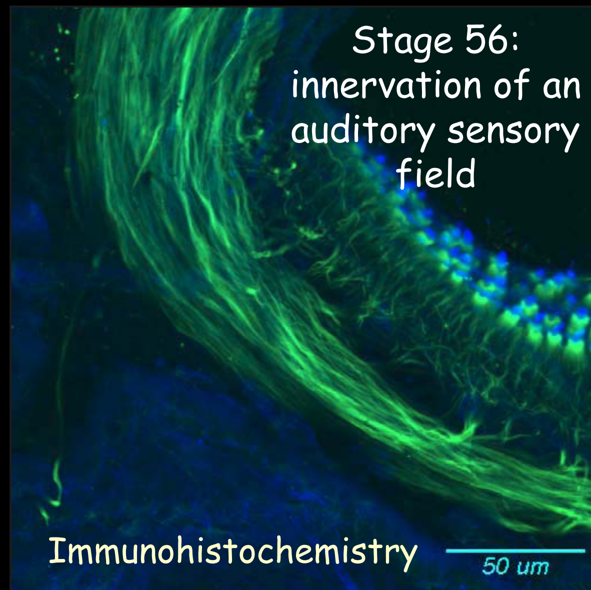
Research Vignette 1

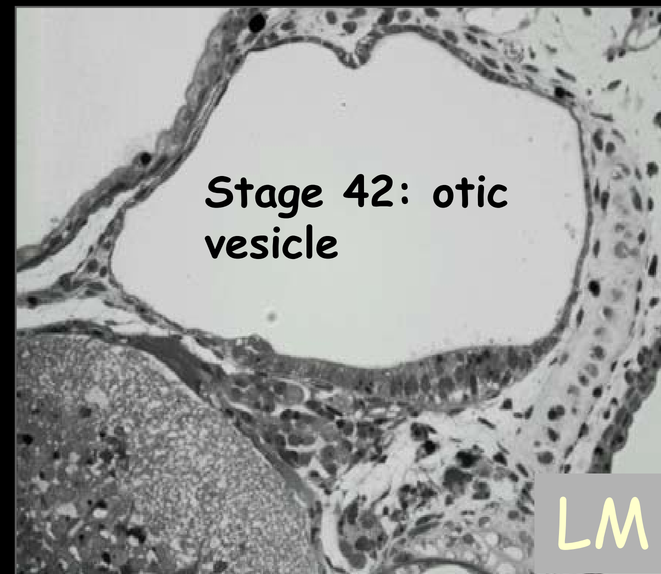
"Classical" Cell Biology

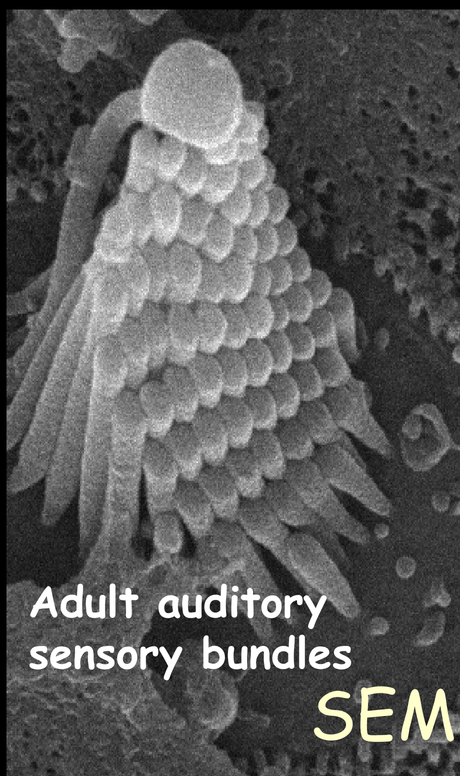
Diversity in the Inner Ear:

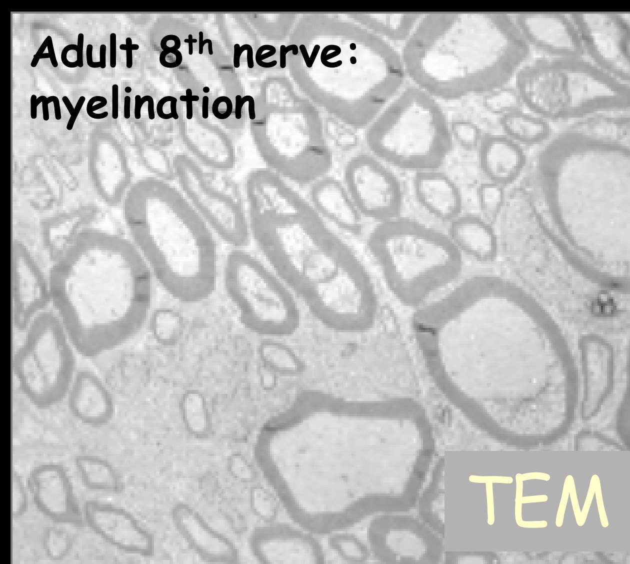
(HAIR) CELLS!!!

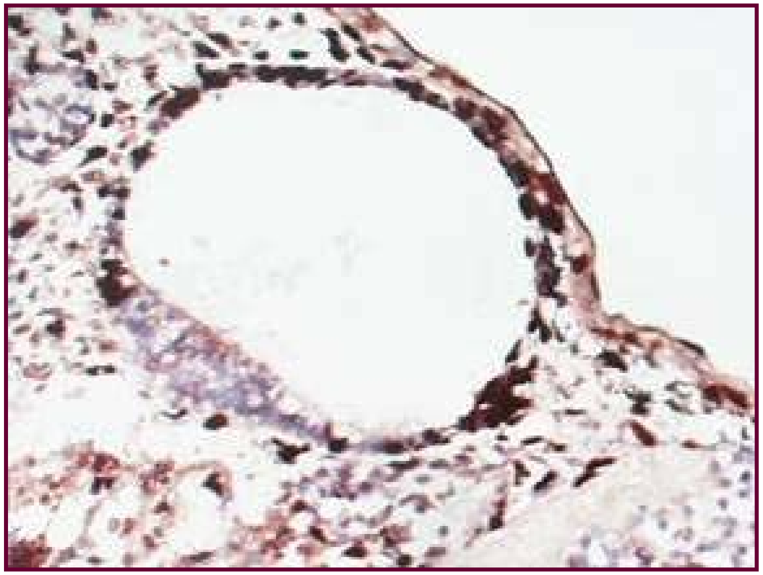
IMAGING THE XENOPUS INNER EAR DURING DEVELOPMENT

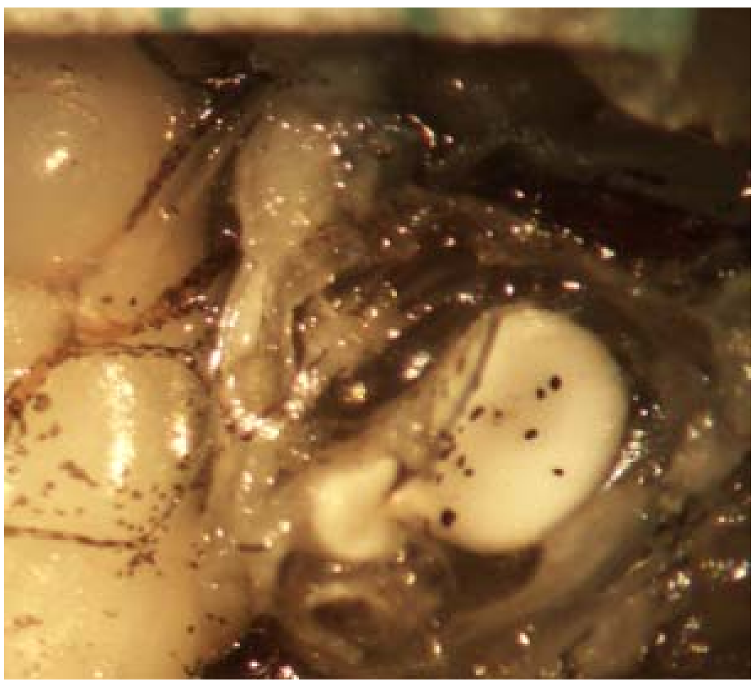






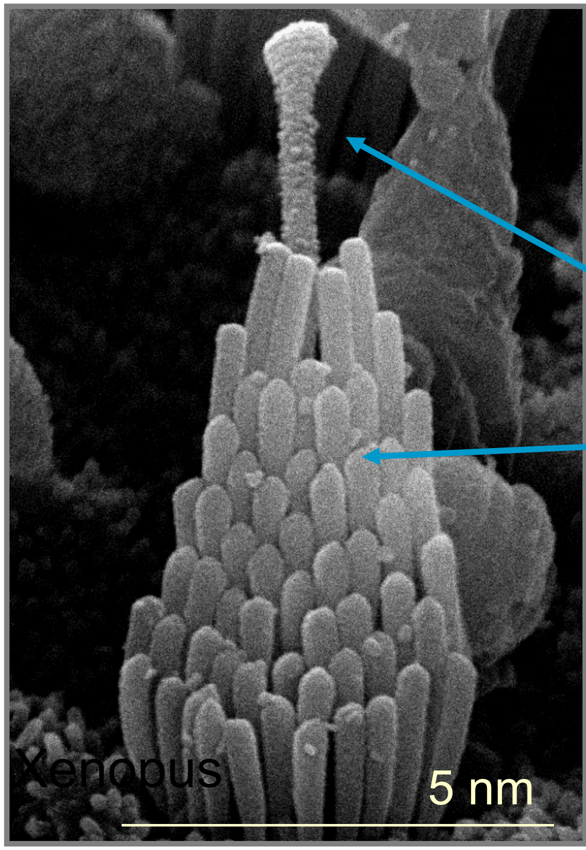






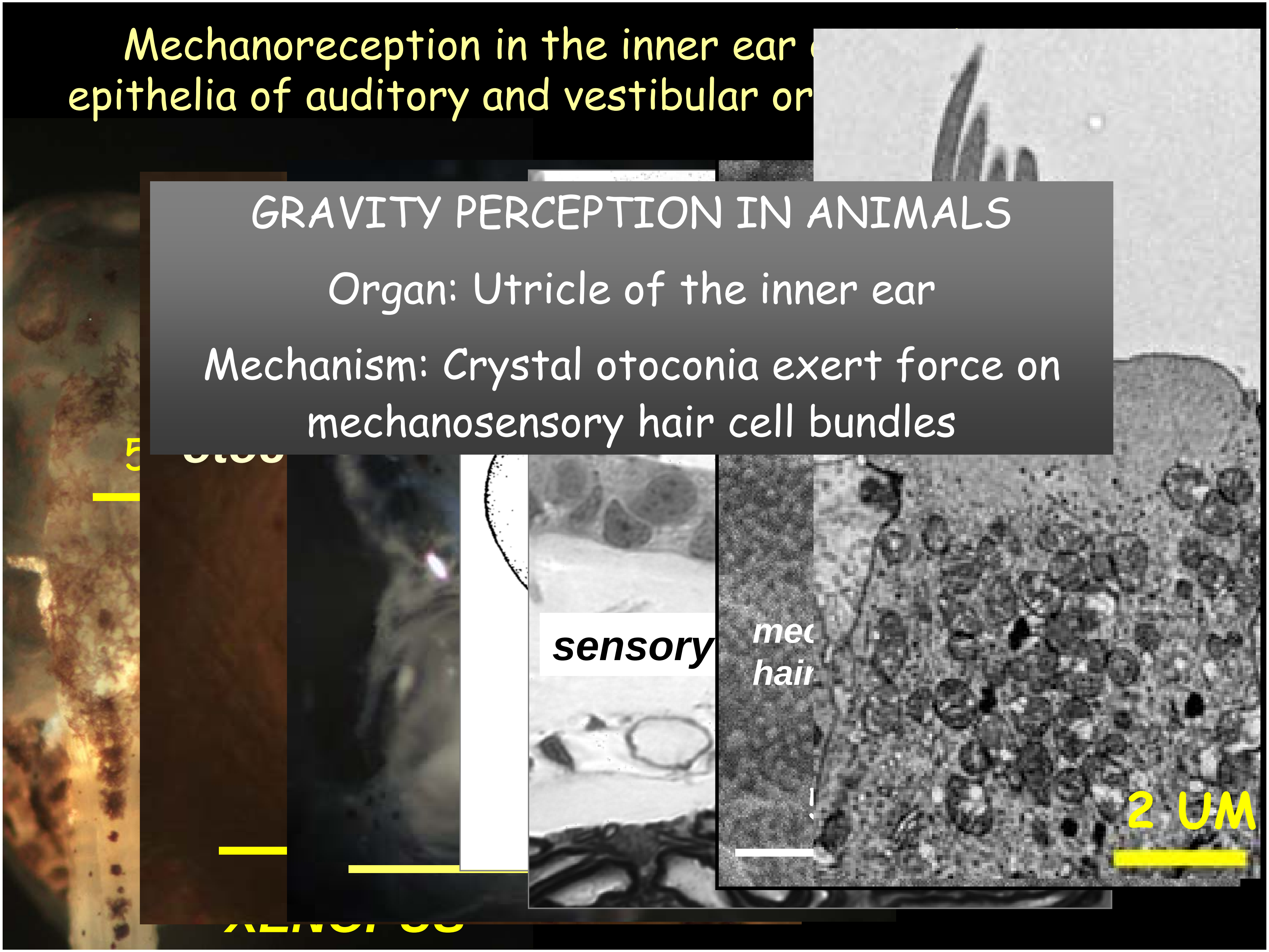
A Tour of *Xenopus* Inner Ear Cells and Organs

The “hair”cell is the common mechanosensory cell in inner ear auditory and vestibular organs

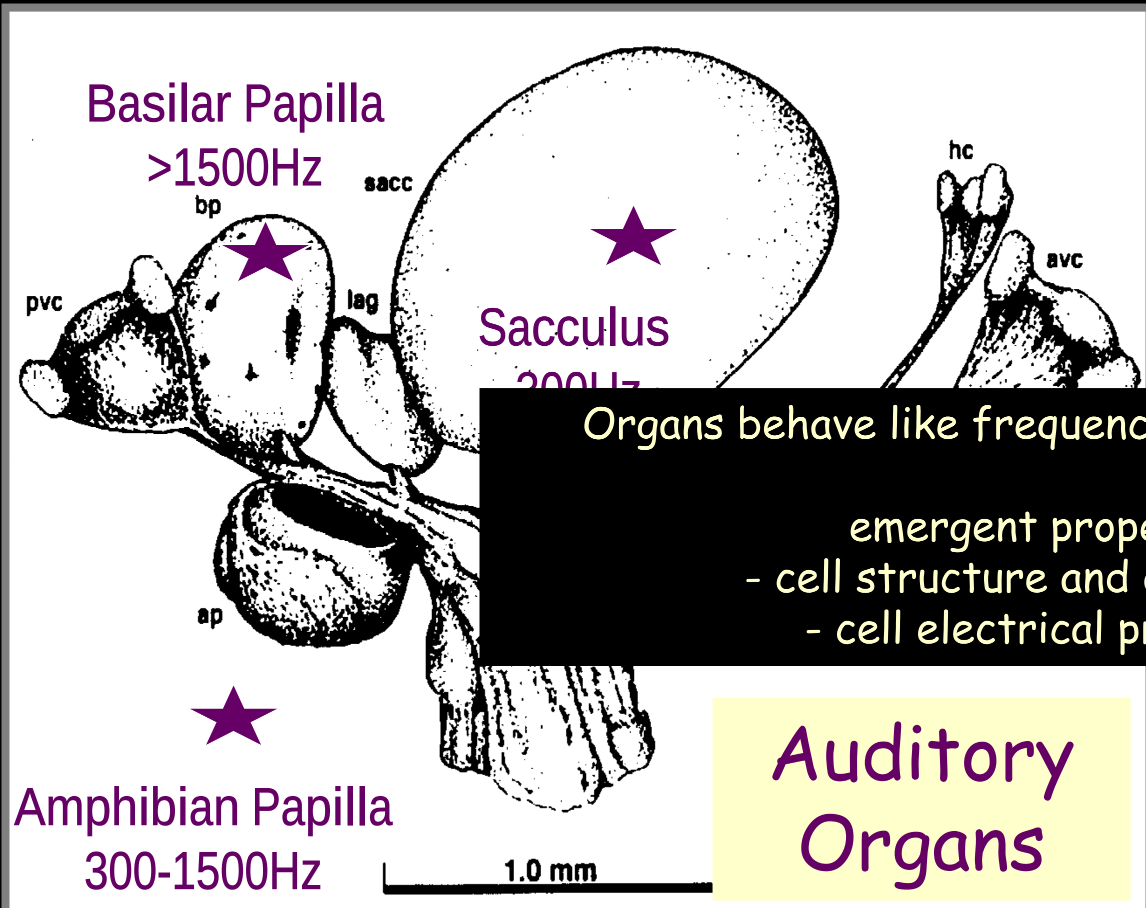


Xenopus **(amphibian) hair cell**

- Kinocilia are filled microtubules
- Stereocilia (villi) are filled with actin microfilaments
- Movement of the bundle initiates mechanoreception
- Damage to these cells can cause loss of vestibular and auditory function


Mechanoreception in the inner ear
epithelia of auditory and vestibular
GRAVITY PERCEPTION IN ANIMALS
Organ: Utricle of the inner ear
Mechanism: Crystal otoconia exert force on
mechanosensory hair cell bundles
sensory
2 UM

The fully formed *Xenopus* inner ear has eight sensory endorgans, each responsive to different frequencies

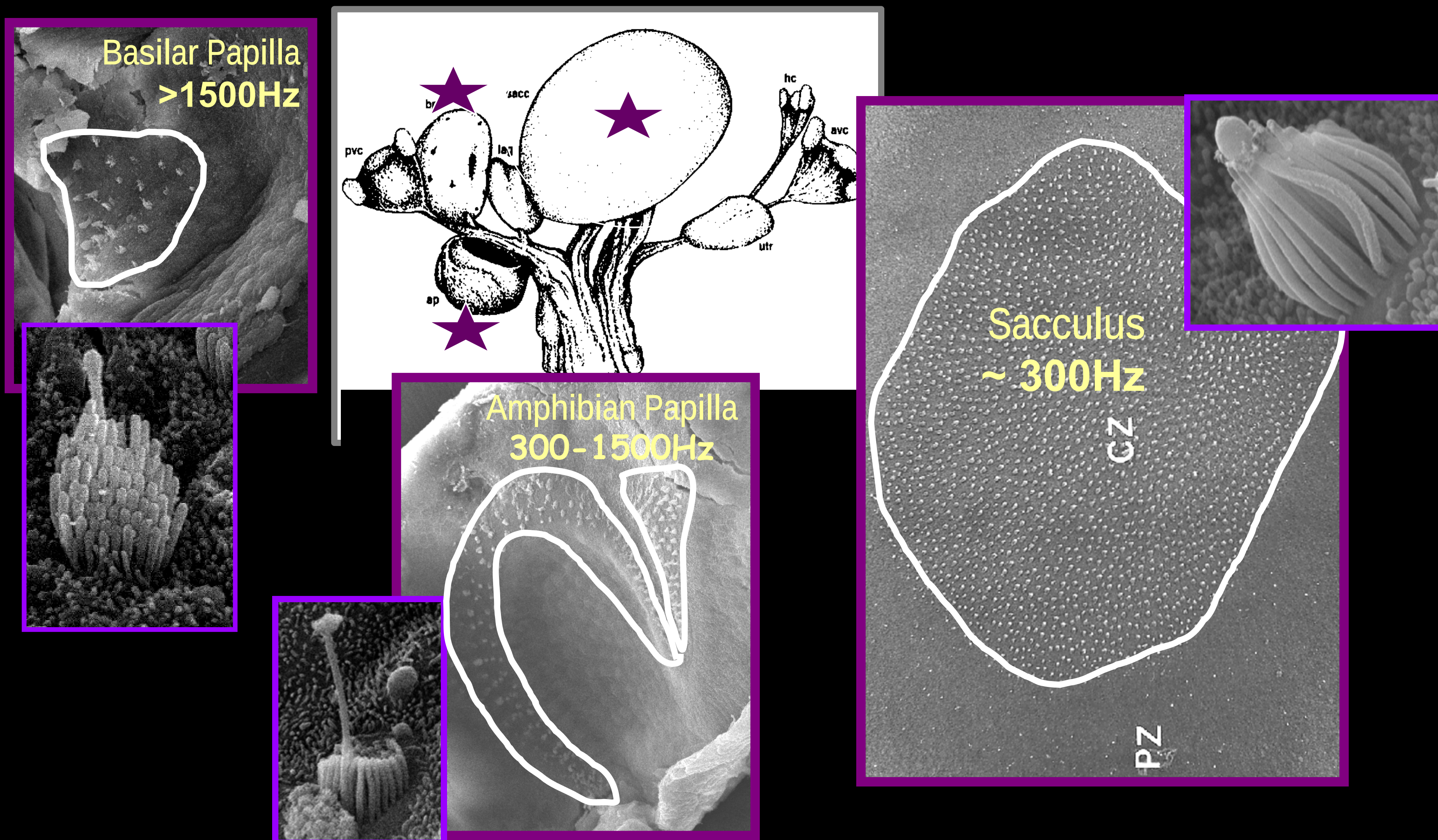


Organs behave like frequency selectivity filters:

emergent property of
- cell structure and organization
- cell electrical properties

2 auditory
5 vestibular
1 acoustico-vestibular
(sacculus)

Endorgans have characteristic sensory field patterns, hair cell numbers and hair cell bundle morphology

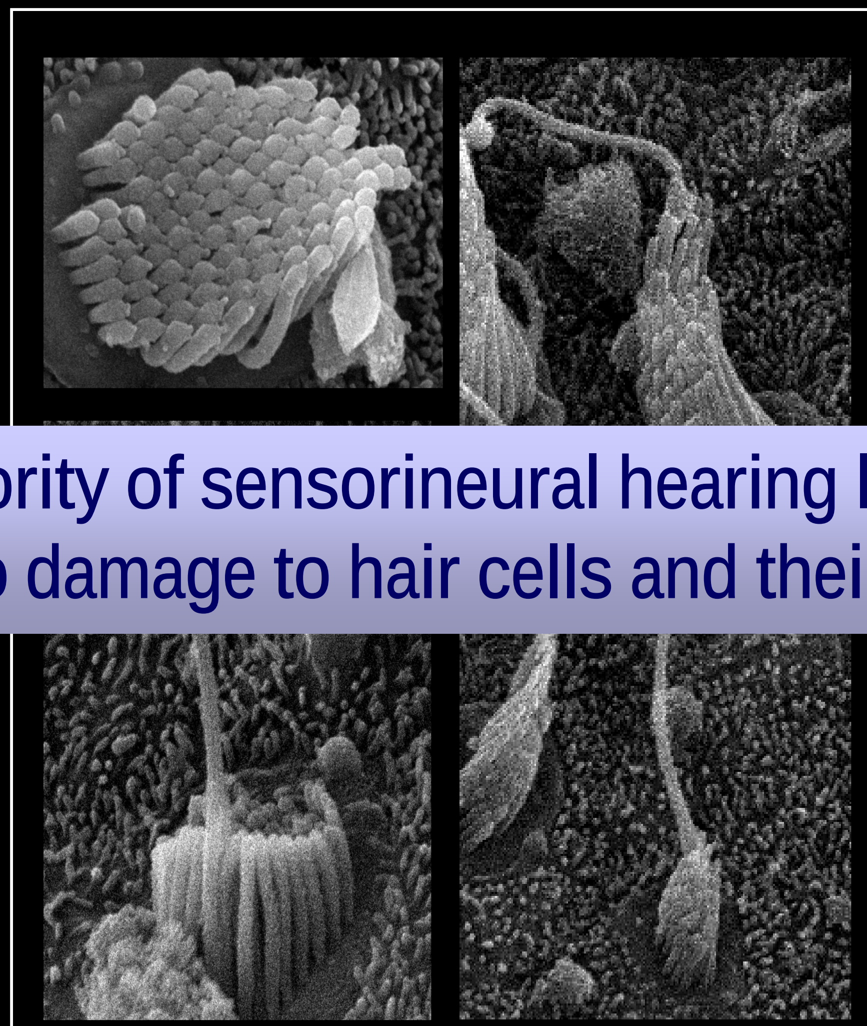


JUVENILE

The morphology of hair cell stereociliary bundles is diverse and characteristic of the sensory organs

SACCULUS

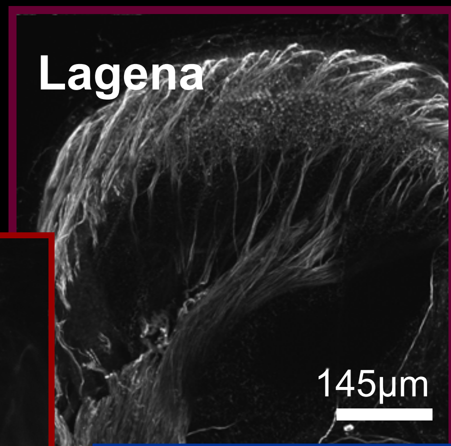
AMPHIBIAN PAPILLA

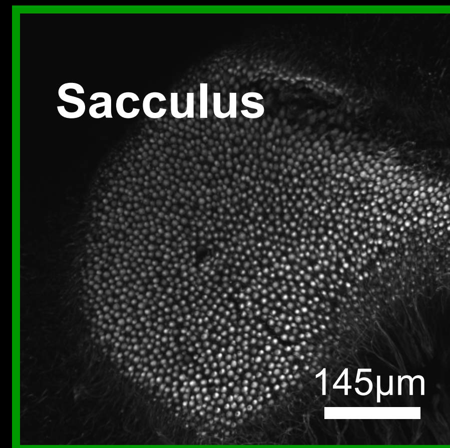


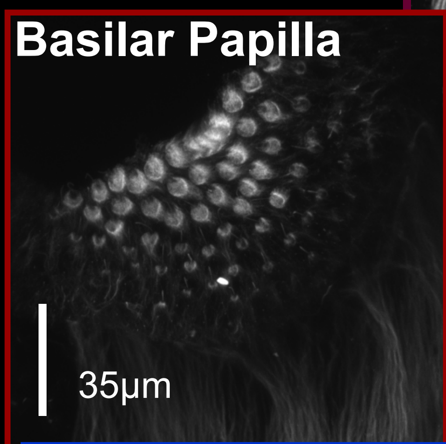
BASILAR PAPILLA

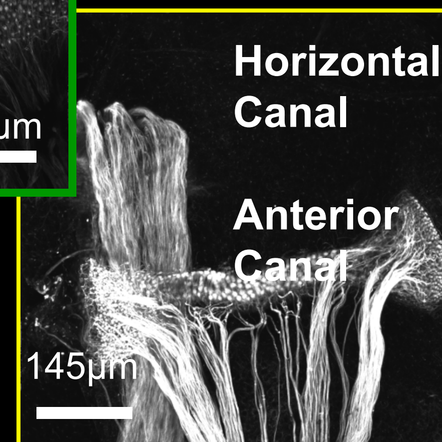
The majority of sensorineural hearing loss is attributed to damage to hair cells and their bundles

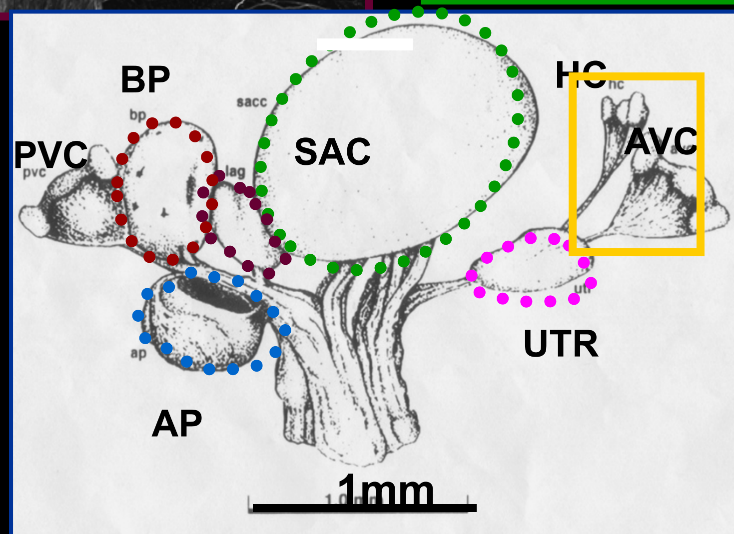
Inner Ear Organogenesis:
Innervation Patterns differ between Endorgans

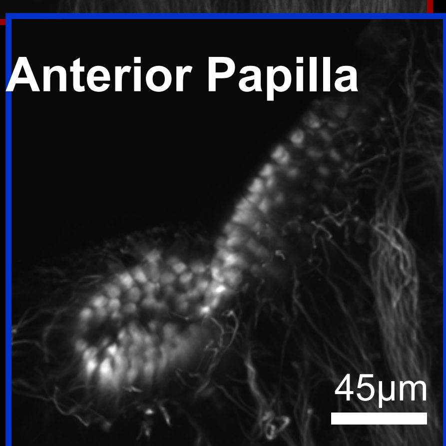


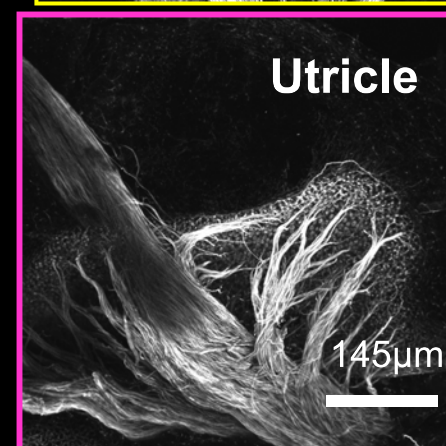












White: Anti-acetylated α-tubulin primary antibody detected with Alexa 488 secondary

STRUCTURAL BIOLOGY

In neuroscience, anatomy is required to provide context for neural pathway analysis

- *Identified structural differences between vestibular and auditory organs*
- *Demonstrated patterns on sensory epithelia as sensory cells acquire mature phenotypes follow different developmental timelines*
- *Provided structural understanding for genetic investigations and modeling of organogenesis and illustrated inner ear complexity*

Research Vignette 2

Diversity in the Inner Ear: Systems Approach

Functional Development: Ion channel expression during organ and sensory field specification

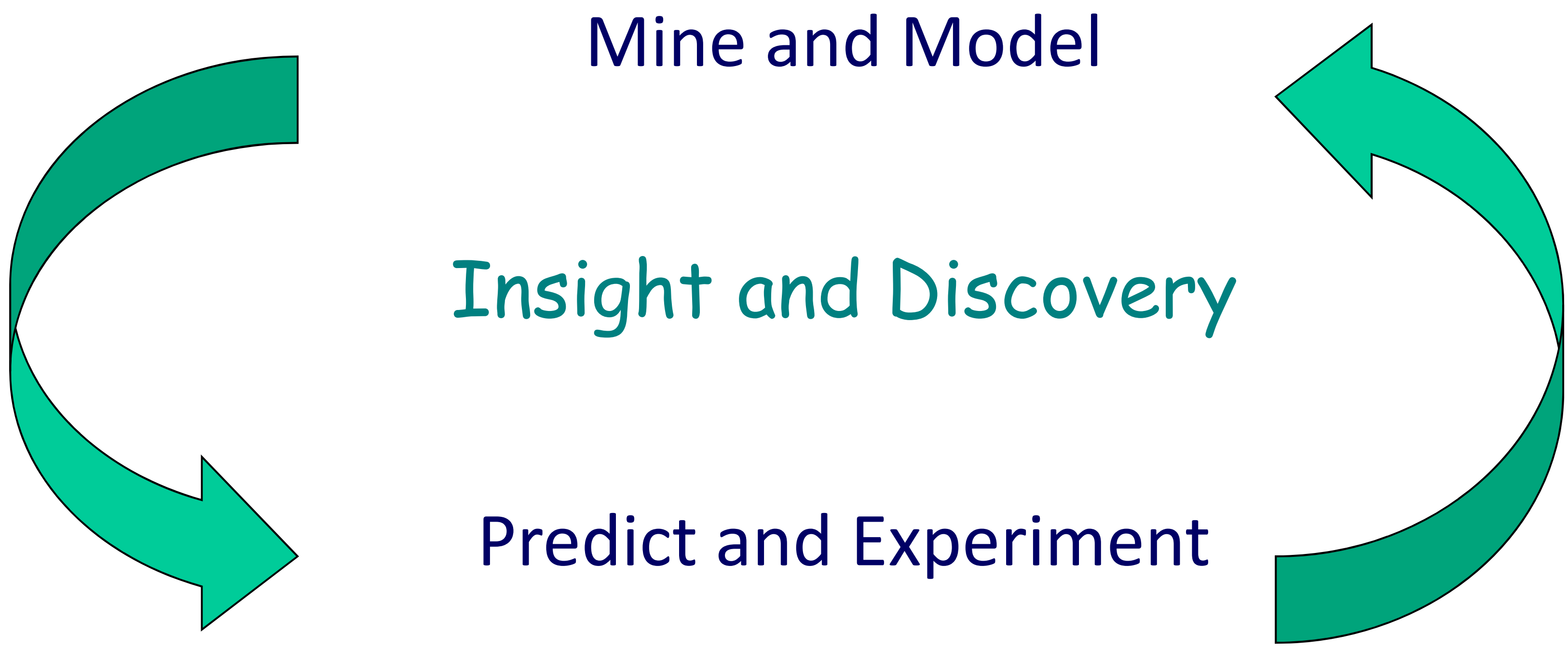
CHALLENGES TO ION CHANNEL RESEARCH

- *Electrophysiology was problematic*
  - *Inaccessible tissue and very few cells at young ages*
  - *Patch clamp difficult to initiate Biology students do not typically have strong quantitative/physics background*
- *Xenopus overtaken by zebrafish, mouse models not feasible, no animal care at NMSU*
- *Ion Channel Gene/In situ detection difficult*
  - *ion channel targets not abundant*
  - *Example: Antibodies for immunodetection not available for Xenopus or signal:noise not acceptable*
  - *"Chosen" gene, BK is one of the most complex in organization*

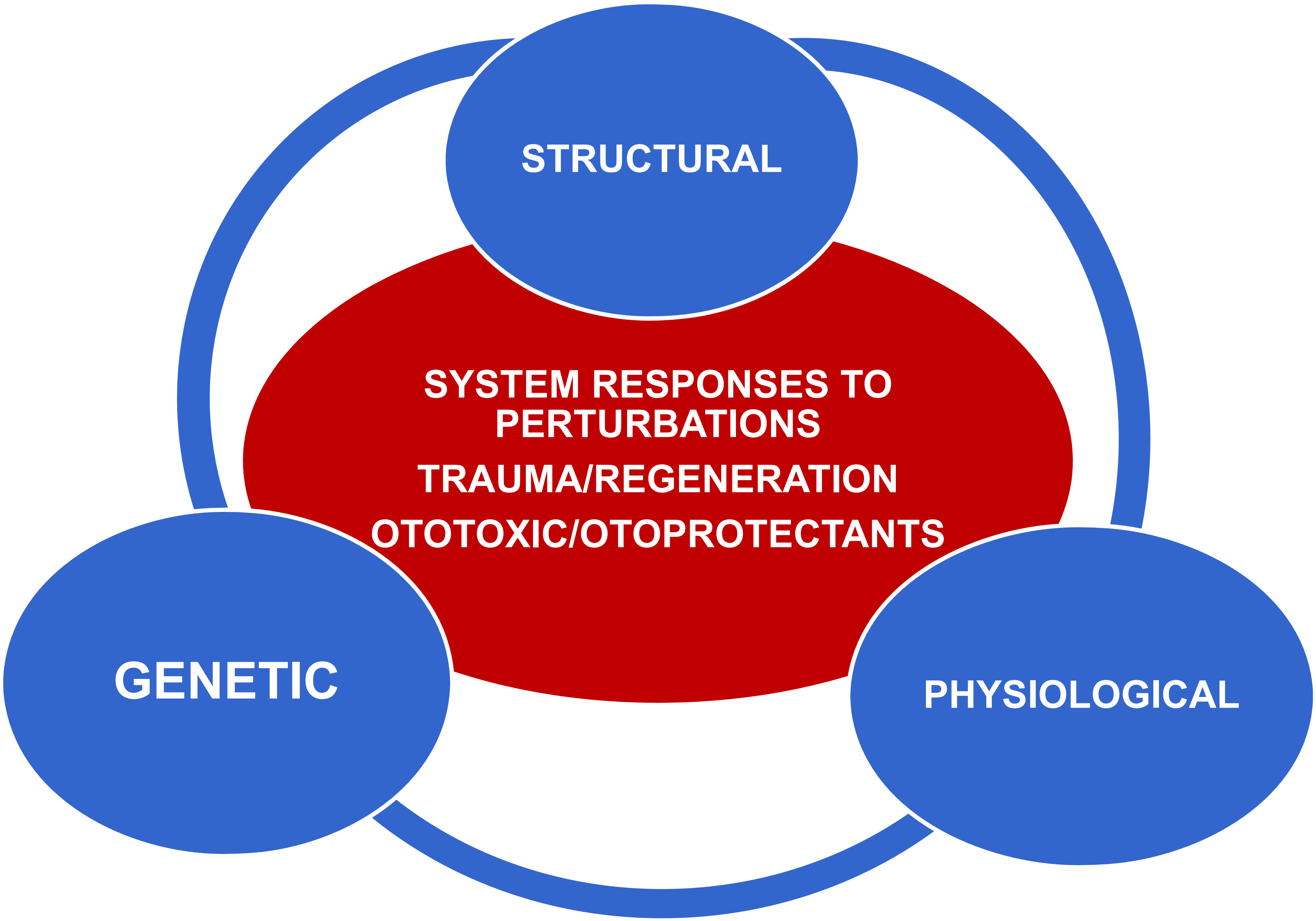
MIT CDP APPROACH
Sorger (Harvard) & Lauffenberger

cell decision processes center

an NIH center of excellence in systems biology



WHAT DATASETS WOULD BE INTEGRATED INTO A KNOWLEDGE MODEL OF ORGANOGENESIS?



NIF

- INFORMATICS-

Heredity:

Organ ***Transcriptional*** Profiling

Therapeutics:

Database of Otoactive Drugs

Mind the Gap

Knowledge Space for Auditory Research

Heredity

What is the inner ear transcriptome and how does it resemble or differ from other organs?

Organ Transcriptomics:

Large Scale Gene Identification Microarrays & RNA Seq

Dr. TuShun Powers

Dr. Daniel Ramirez-Gordillo

Casilda Trujillo-Provencio

Dr. Selene Virk

V. Bleu Knight

Dr. Charlie Whittaker

Manlin Luo

Jennifer van Velkinburgh

Faye Schilkey

Experimental Strategies for Inner Organ RNA-Seq/Microarray Data Analysis

1. Identify genes important for inner ear function
   - ➢ link to probe set IDs (PSIDs) on the *X. laevis* GeneChip®
   - ➢ Map to Xt and Xl genomes (RNA Seq)
2. Comparative organ/species analysis
   - Increase success of semantic queries
     - *Annotation linkage to informative identifiers*
   - Sequence similarity mapping to human proteins (OMIM genes for deafness and vestibular dysfunction)

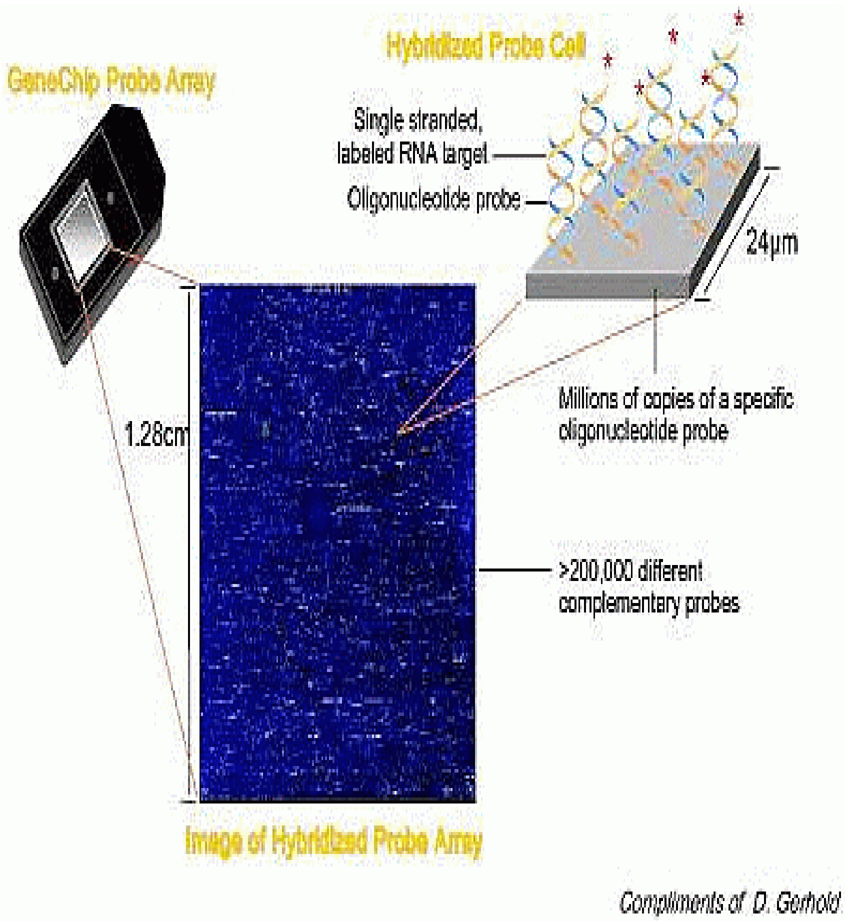
High Throughput Transcriptomics

Microarray

- Known sequences arrayed on chip
- Hybridization reaction detects homology of unknown seq with probe set
- Large files
- Statistical analysis
- Annotated sequences

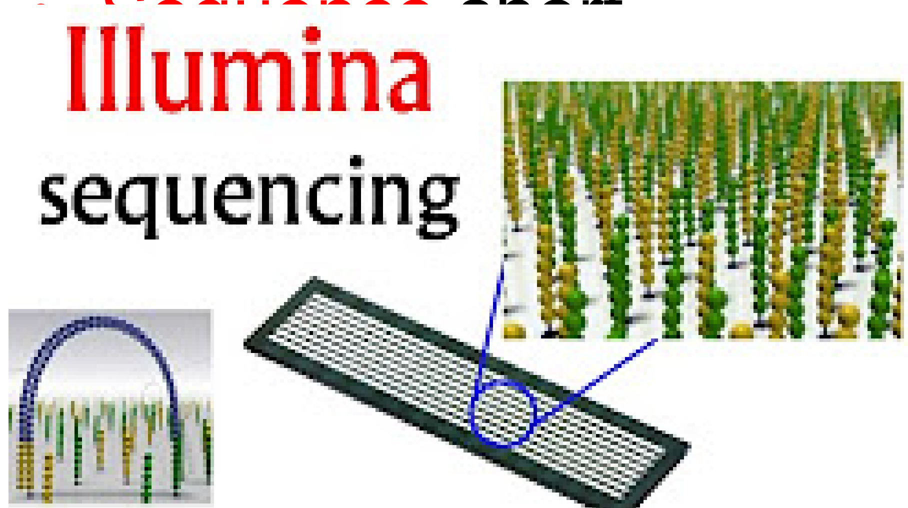
- RNA seq
- Sequence short fragments
- Map to reference genome
- Very large files
- Assembled genome
- Statistical analysis

High Throughput Transcriptomics



- Annotated sequences

- RNA seq

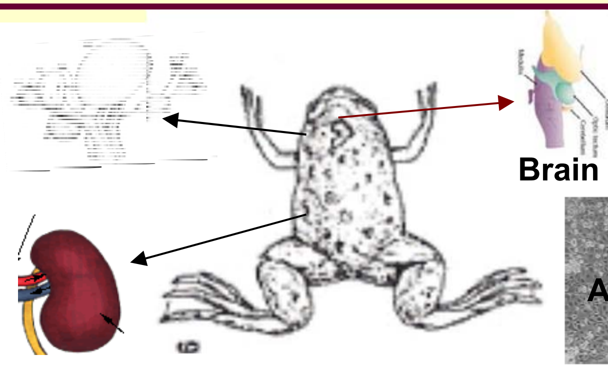


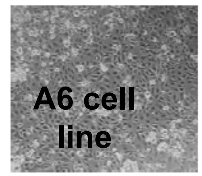
- Assembled genome
- Statistical analysis

Probing Organ Decision Processes
RNA-Seq and Microarray Analysis

Tissue Collection

Larval and Juvenile *Xenopus laevis*

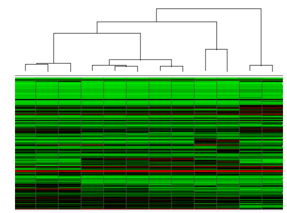




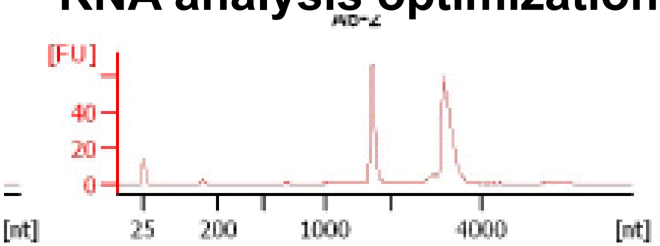
Transcriptomic Analysis Platforms

Alpheus. JMP, R

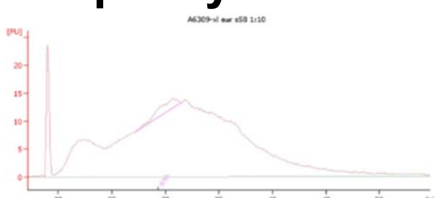
Bioconductor



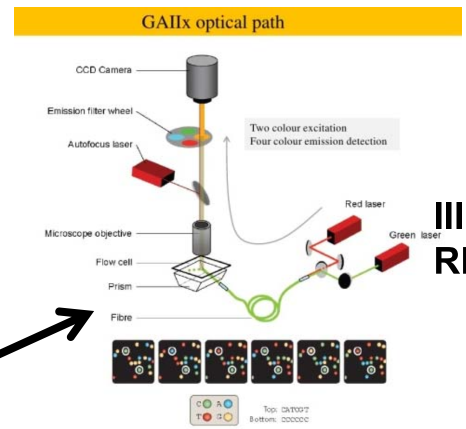
RNA analysis optimization



cRNA quality



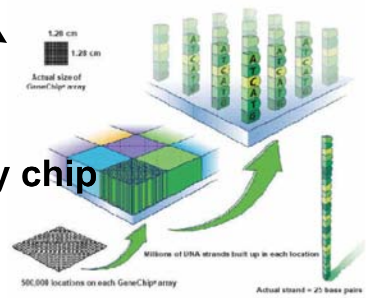
cDNA



Illumina RNA Seq

Image files

Xenopus Affy chip



Example of Systems Insight from Organ RNA Profiling

1. Transporters expressed in inner ear dominated by kidney transporters not MHC channels

    - Ion channels important for organ *sensory* task (sense perception) but organ *integrity* relies on transporters common to the kidney

2. Differential gene expression
    - *greatest kidney vs brain*
    - *can identify inner ear centric genes: COL2A1 & GJB2*

3. Comparative organ transcriptomics uncovers signature "***channelome***" of inner ear, brain and kidney (potential drug targets)

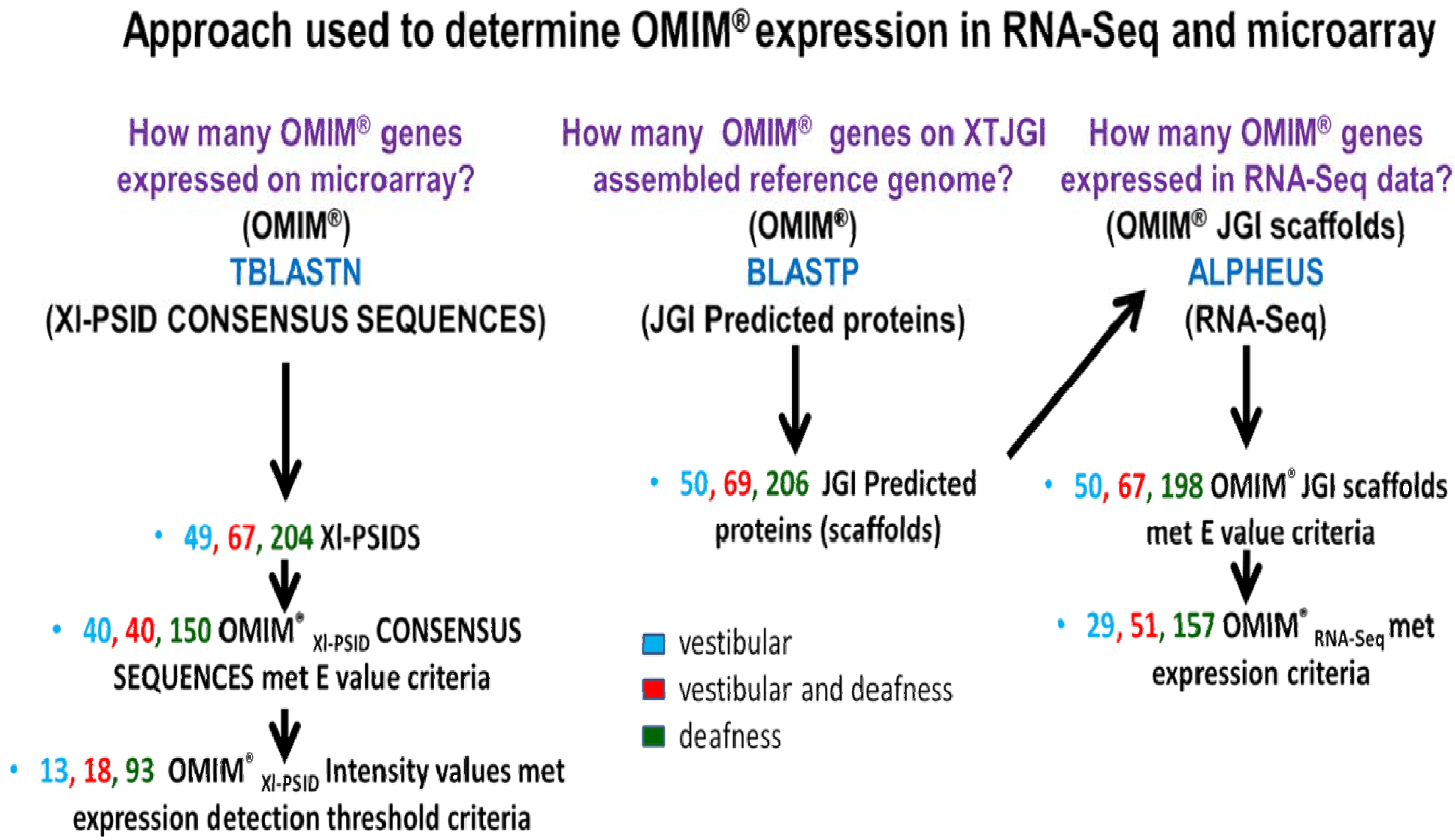
Heredity

Can Xenopus orthologues for OMIM deafness and vestibular disorders be identified?

Relevance of animal models

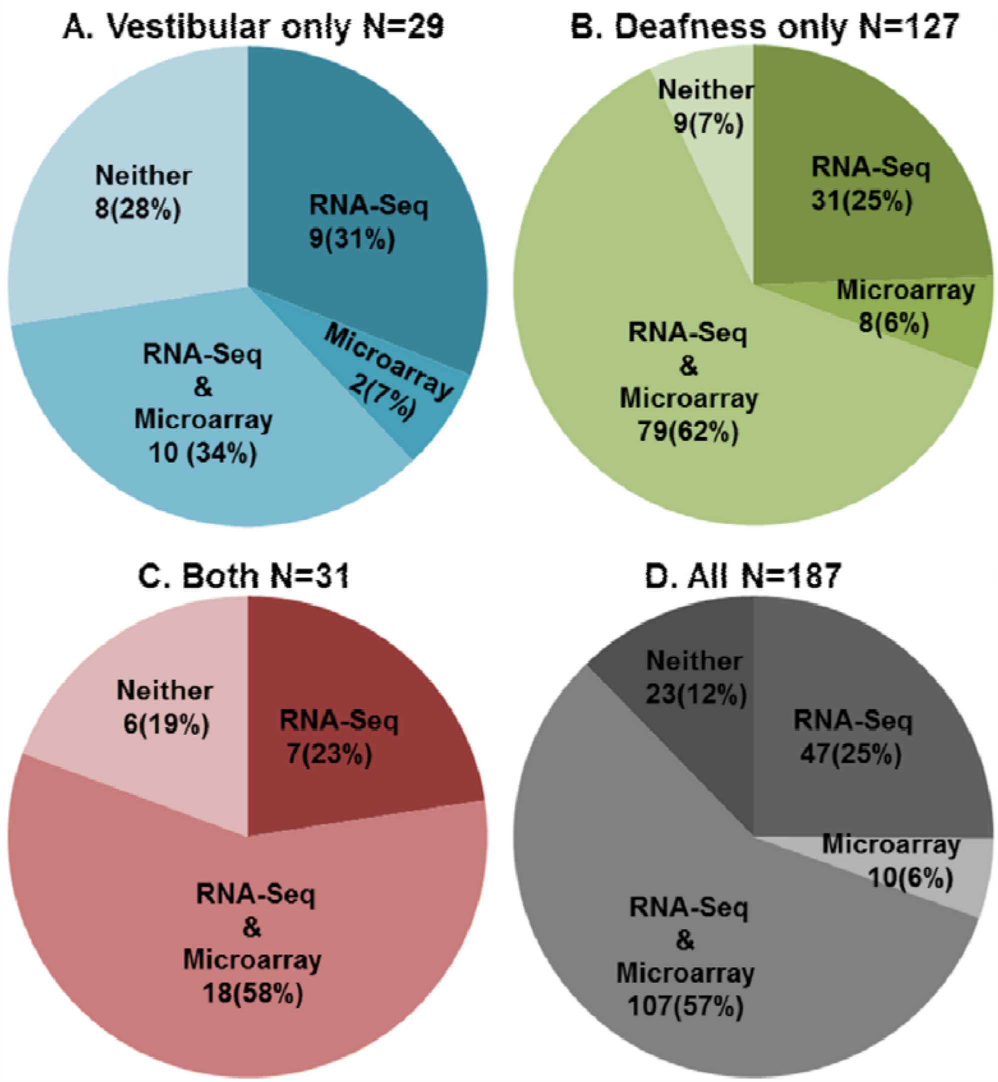
Informatics / Microarray & RNA Seq

Curate list of OMIM genes



http://bmcresnotes.biomedcentral.com/articles/10.1186/s13104-015-1485-1

Insight: Xenopus OMIM orthologues
The two technologies combined identify more genes



http://bmcresnotes.biomedcentral.com/articles/10.1186/s13104-015-1485-1

Identified target regions on *Xenopus tropicalis* scaffolds for genome editing experiments and mutational analysis

Scaffold information for Xenopus OMIM channel and transporters orthologues implicated in deafness and vestibular disorders

Perfect timing with Woods Hole *Xenopus* facility

http://www.mbl.edu/xenopus/

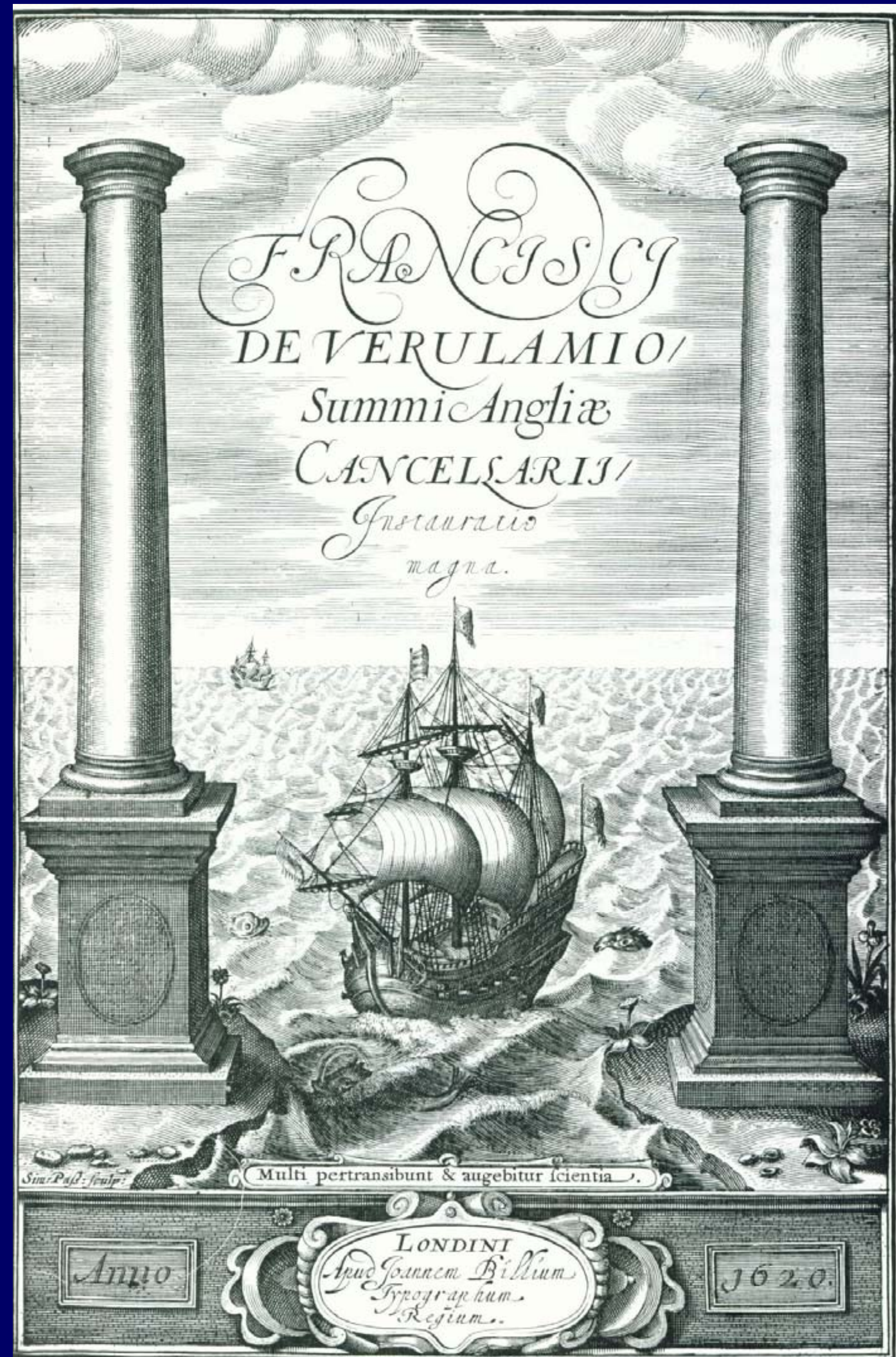
Professor Quincy Quick

Professor Marti Morales

Professor Shannon Manuelito

Dr. Ramirez-Gordillo

Plus Ultra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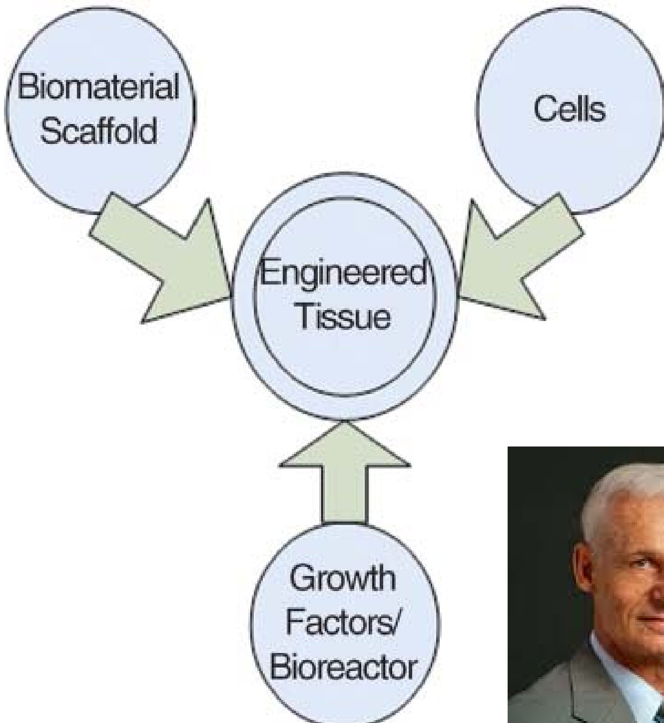
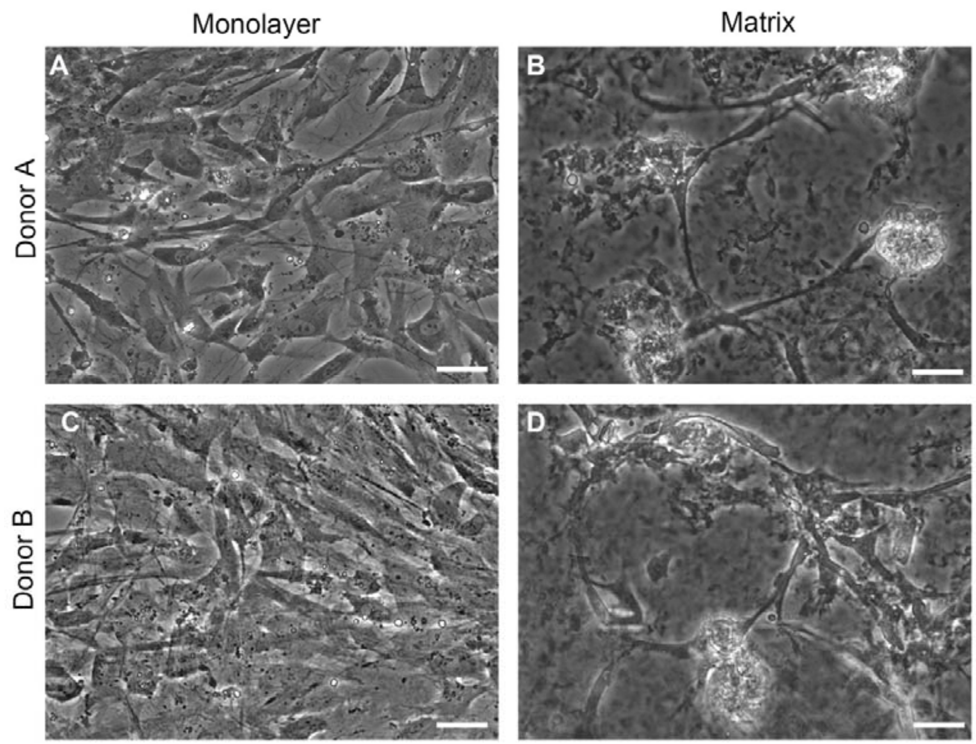
Neural Tissue Engineering Biomechanics

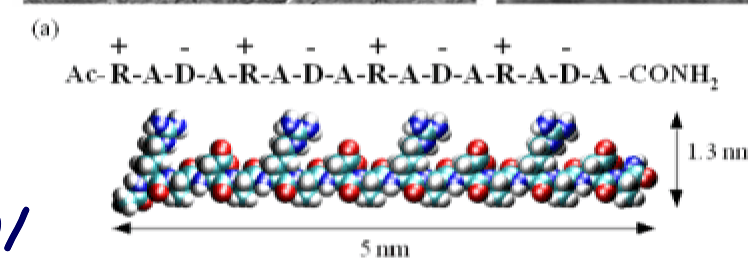
Manasi Jogalekar

V. Bleu Knight



BRUCE RANSOM





https://peerj.com/articles/2829/

CONCLUSIONS

Concluding Thoughts about Informatics

- Large scale analysis gives amore impartial eye view and highlights (human) bias in knowledge production
- Neuroscientists, especially biophysicists/electrophysiologists, are well positioned to work in this area to define informatics questions
- Data science can be a great democratizer but the senior faculty will need to retrain
- High throughput methods and mass production, high-tech wet labs make it very difficult for traditional small labs, especially in poor states, to compete. Informatics/data science must be pursued
- *Future of science? data generators and data analyzers?*

Insight for Biology Education

Mathematical reasoning and statistics must be integrated into the biology curriculum at all levels, in all courses

Gratitude

- Mentors for their patience and integrity
- colleagues and extraordinary students at NMSU
  - Student Training Programs: NMSU Honors College Crimson Scholars, NIH R25 (MBRS RISE/BPENDURE)
- NIH R03, R01, P50
- NSF MRI awards
- NASA
- Whitehall
- CINT

Sometimes, you have to back-pedal to go forward

~ E. Izzard